PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
AMENDMENTS OF THE CLAIMS

(PCT Rule 62 and
Administrative Instructions, Section 417)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as International Preliminary Examining Authority

Date of mailing (day/month/year)	
01 October 2001 (01.10.01)	
International application No.	International filing date (day/month/year)
PCT/IL99/00441	09 September 1999 (09.09.99)

Applicant

HADASIT MEDICAL RESEARCH SERVICES AND DEVELOPMENT COMPANY LTD. et al

The International Bureau hereby informs the International Preliminary Examining Authority that no amendments under Article 19 have been received by the International Bureau (Administrative Instructions, Section 417).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Anne KARKACHI

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 01 October 2001 (01.10.01)	
International application No. PCT/IL99/00441	**Applicant's or agent's file reference** 959/39
International filing date (day/month/year) 09 September 1999 (09.09.99)	**Priority date** (day/month/year)
Applicant PINES, Mark et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

05 March 2001 (05.03.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Anne KARKACHI

Telephone No.: (41-22) 338.83.38

PA NT COOPERATION TREAT

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF DEFECTS IN DEMAND

(PCT Rule 60.1(d))

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE
in its capacity as International Preliminary Examining Authority

Date of mailing (day/month/year)	01 October 2001 (01.10.01)
International application No.	PCT/IL99/00441
International filing date (day/month/year)	09 September 1999 (09.09.99)

Applicant: HADASIT MEDI(NT COMPANY LTD. et al

The International Bureau hereby r IL 99 that it has found that the demand is defective for the reasons indicate

1. ☐ It does not contain the el · ll (Rule 53.2(a)(iv) and 53.7).
2. ☐ It does not permit the ide tes (Rule 60.1(b)).
3. ☐ It does not contain the re
4. ☐ It does not contain the re ne Annex (Rules 53.2(a)(ii) and 53.5).
5. ☐ It does not contain the r on as specified in the Annex (Rules 53.2(a)(iii) and 53
6. ☐ It is not submitted in the _____ (Rule 55.1).
7. ☐ It is not made on the printed form (Rule 53.1(a)).
8. ☐ It is presented as a computer print-out the particulars of which do not comply with the Administrative Instructions (Rule 53.1(a)).
9. ☒ It does not contain the required indications concerning the applicant as specified in the Annex (Rules 53.2(a)(ii) and 53.4).
10. ☐ It does not contain the required signature as specified in the Annex (Rules 53.2(b) and 53.8).

Other observations, if necessary:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorised officer
Anne KARKACHI

Telephone No.: (41-22) 338.83.38

NOTIFICATION OF DEFECTS IN DEMAND

International application No.
PCT/IL99/00441

Continuation of item 4: As to indications concerning **the agent** (Rule 4.4), the demand:

a. ☐ does not properly indicate the agent's name (specify):

b. ☐ does not indicate the agent's address.

c. ☐ does not properly indicate the agent's address (specify):

Continuation of item 5: As to indications concerning **the international application**, the demand does not indicate:

a. ☐ the international filing date.

b. ☐ the international application number.

c. ☐ the name of the receiving Office, where the international application number was not known to the applicant at the time the demand was filed.

d. ☐ the title of the invention.

Continuation of item 9: As to indications concerning **the applicant** (Rules 4.4 and 4.5), the demand:

a. ☐ does not indicate all the applicants for the elected States.

b. ☒ does not properly indicate the applicant's name (specify): Hadasit Medical Services... or Hadasit Medical Research...

c. ☐ does not indicate the applicant's address.

d. ☐ does not properly indicate the applicant's address (specify):

e. ☐ does not indicate the applicant's nationality.

f. ☐ does not indicate the applicant's residence.

Continuation of item 10: As to requirements concerning **signature** (Rules 4.15 and 90.4), the demand:

a. ☐ is not signed.

b. ☐ is not signed by all the applicants for the elected States.

c. ☐ is not accompanied by the statement referred to in the check list in Box No. VI of the demand explaining the lack of the signature of an applicant for the election of the United States of America.

d. ☐ is signed by what appears to be an agent/common representative but

☐ the demand is not accompanied by a power of attorney appointing him.

☐ the power of attorney accompanying the demand is not signed by all the applicants for the elected States.

Copy for the designated Office (DO/US)

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

COMMUNICATION IN CASES FOR WHICH NO OTHER FORM IS APPLICABLE

To:
FRIEDMAN, Mark, M.
Beit Samueloff
Haomanim Street 7
67897 Tel Aviv
ISRAËL

Date of mailing (*day/month/year*) 13 October 1999 (13.10.99)	
Applicant's or agent's file reference 959/39	**REPLY DUE** see paragraph 1 below
International application No. PCT/IL99/00441	International filing date (*day/month/year*) 09 September 1999 (09.09.99)
Applicant HADASIT MEDICAL SERVICES AND DEVELOPMENT COMPANY LTD.	

1. ☐ REPLY DUE within _____ months/days from the above date of mailing

☐ NO REPLY DUE, however, see below

☒ IMPORTANT COMMUNICATION

☐ INFORMATION ONLY

2. COMMUNICATION:

The applicant, in respect of the above-identified international application, is notified that pursuant to the provisions of PCT Article 14(2) and PCT Rule 20.2 the receiving Office (RO/IL) has informed the International Bureau (WO) that the International Filing Date should be changed to indicate 09 September 1999 (09.09.99) instead of 13 August 1998 (13.08.98).

A copy of this notification has been sent to the receiving Office (RO/IL), the International Searching Authority (ISA/US) and the designated Offices already notified of their designation.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Patricia Gonzalez
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Form PCT/IB/345 (July 1992)

Copy for the International Searching Authority (ISA/US)

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

COMMUNICATION IN CASES FOR WHICH NO OTHER FORM IS APPLICABLE

To:

FRIEDMAN, Mark, M.
Beit Samueloff
Haomanim Street 7
67897 Tel Aviv
ISRAËL

Date of mailing (*day/month/year*) 13 October 1999 (13.10.99)	
Applicant's or agent's file reference 959/39	**REPLY DUE** see paragraph 1 below
International application No. PCT/IL99/00441	International filing date (*day/month/year*) 09 September 1999 (09.09.99)
Applicant HADASIT MEDICAL SERVICES AND DEVELOPMENT COMPANY LTD.	

1. ☐ REPLY DUE within _____ months/days from the above date of mailing

☐ NO REPLY DUE, however, see below

☒ IMPORTANT COMMUNICATION

☐ INFORMATION ONLY

2. COMMUNICATION:

The applicant, in respect of the above-identified international application, is notified that pursuant to the provisions of PCT Article 14(2) and PCT Rule 20.2 the receiving Office (RO/IL) has informed the International Bureau (WO) that the International Filing Date should be changed to indicate 09 September 1999 (09.09.99) instead of 13 August 1998 (13.08.98).

A copy of this notification has been sent to the receiving Office (RO/IL), the International Searching Authority (ISA/US) and the designated Offices already notified of their designation.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Patricia Gonzalez
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Form PCT/IB/345 (July 1992)

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: MARK M. FRIEDMAN
C/O CASTORINA, ANTHONY
2001 JEFFERSON DAVIS HIGHWAY
SUITE 207
ARLINGTON, VIRGINIA 22202

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	27 SEP 2001

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
959/39	

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/IL99/00441	09 SEPTEMBER 1999	NONE

Applicant

HADASIT MEDICAL RESEARCH SERVICES AND DEVELOPMENT COMPANY LTD.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	FREDERICK KRASS
Facsimile No. (703) 305-3230	Telephone No. (703) 308-1235

Form PCT/IPEA/416 (July 1992)*

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 959/39	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/IL99/00441	International filing date *(day/month/year)* 09 SEPTEMBER 1999	Priority date *(day/month/year)* NONE
International Patent Classification (IPC) or national classification and IPC IPC(7): A61K 31/505 and US Cl.: 514/59		
Applicant HADASIT MEDICAL RESEARCH SERVICES AND DEVELOPMENT COMPANY LTD.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05 MARCH 2001	07 SEPTEMBER 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer FREDERICK KRASS Telephone No. (703) 308-1235

Form PCT/IPEA/409 (cover sheet) (July 1998) *

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/IL99/00441

I. Basis of the report

1. With regard to the elements of the international application:*

[X] the international application as originally filed

[X] the description:

pages 1-21
pages NONE , as originally filed
pages NONE , filed with the letter of , filed with the demand

[X] the claims:

pages 22-26
pages NONE , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the letter of , filed with the demand

[X] the drawings:

pages 1-10
pages NONE , as originally filed
pages NONE , filed with the letter of , filed with the demand

[X] the sequence listing part of the description:

pages NONE
pages NONE , as originally filed
pages NONE , filed with the letter of , filed with the demand

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE
[X] the claims, Nos. NONE
[X] the drawings, sheets/fig NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINAR[illegible] EXAMINATION REPORT

International application No.
PCT/IL99/00441

V. Reasoned statement under Article 3[illegible](2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporti[illegible]g such statement

1. statement

Novelty (N)	Claims	1-18	YES
	Claims	NONE	NO
Inventive Step (IS)	Claims	1-18	YES
	Claims	NONE	NO
Industrial Applicability (IA)	Claims	1-18	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-18 meet the criteria set [illegible]t in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest compositions comprising halofuginone which are specifically intended for use in wound healing, stricture treatment, or the prevention of cicatrix formation, nor such methods of use.

Claims 1-18 meet the criteria set [illegible]t in PCT Article 33(4); the applicability of the claimed compositions and methods to the pharmaceutical/medical industries is self-evident.

---------- NEW CITATIONS ------ ----------
NONE

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL99/00441

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :A61K 31/505
US CL :514/259
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/259

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - A	US 5,891,879 A (NAGLER et al.) 06 April 1999 (6/4/99), see the entire document, especially column 3, lines 3-20.	1-11, 18 --- 12-17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
03 MAY 2000	13 JUN 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer FREDERICK KRASS Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)*

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 02 OCT 2001
WIPO PCT

Applicant's or agent's file reference 959/39	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/IL99/00441	International filing date *(day/month/year)* 09 SEPTEMBER 1999	Priority date *(day/month/year)* NONE
International Patent Classification (IPC) or national classification and IPC IPC(7): A61K 31/505 and US Cl.: 514/259		
Applicant HADASIT MEDICAL RESEARCH SERVICES AND DEVELOPMENT COMPANY LTD.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05 MARCH 2001	07 SEPTEMBER 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer FREDERICK KRASS Telephone No. (703) 308-1235

Form PCT/IPEA/409 (cover sheet) (July 1998) ★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/IB99/00441

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-21 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the claims:
pages 22-26 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the drawings:
pages 1-10 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/[illegible]99/00441

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-18	YES
	Claims	NONE	NO
Inventive Step (IS)	Claims	1-18	YES
	Claims	NONE	NO
Industrial Applicability (IA)	Claims	1-18	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-18 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest compositions comprising halofuginone which are specifically intended for use in wound healing, stricture treatment, or the prevention of cicatrix formation, nor such methods of use.

Claims 1-18 meet the criteria set out in PCT Article 33(4); the applicability of the claimed compositions and methods to the pharmaceutical/medical industries is self-evident.

---------- NEW CITATIONS ----------
NONE

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 March 2001 (15.03.2001)

PCT

(10) International Publication Number
WO 01/17531 A1

(51) International Patent Classification[7]: A61K 31/505

(21) International Application Number: PCT/IL99/00441

(22) International Filing Date:
9 September 1999 (09.09.1999)

(25) Filing Language: English

(26) Publication Language: English

(71) Applicant *(for all designated States except US)*: **HADASIT MEDICAL RESEARCH SERVICES AND DEVELOPMENT COMPANY LTD.** [IL/IL]; Kiryat Hadassah, 91120 Jerusalem (IL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PINES, Mark** [IL/IL]; Pinsker Street 12B, 76308 Rehovot (IL). **VLODAVSKY, Israel** [IL/IL]; Arbel Street 34, 90805 Mevasseret Zion (IL). **NAGLER, Arnon** [IL/IL]; Sderot Herzl 46, 74381 Jerusalem (IL).

(74) Agent: **FRIEDMAN, Mark, M.**; Beit Samueloff, Haomanim Street 7, 67897 Tel Aviv (IL).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/17531 A1

(54) Title: PROMOTION OF WOUND HEALING

(57) Abstract: A promotor of wound healing and an inhibitor of formation of a urethral stricture, particularly following surgical intervention or infection in the area. Specifically, the most preferred compound of the present invention, Halofuginone, can be used to prevent collagen deposition from occurring within the lumen of the urethra after such trauma, thereby inhibiting urethral stricture formation. Halofuginone, and related compounds, are also useful for the promotion of wound healing after trauma, for example after surgery.

PROMOTION OF WOUND HEALING

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to a composition and a method for the promotion of wound healing, and. in addition, to a composition and a method for the prevention of the formation of strictures, such as urethral strictures.

Wound healing is a complex process involving such factors as cells, extracellular matrix (ECM) components and the cellular microenvironment. Essentially, all wound healing involves the repair or replacement of damaged tissues. The precise nature of such repair or replacement depends upon the tissues involved, although all such processes involve certain basic principles. To illustrate these principles, cutaneous, or skin, wound healing will be described, it being understood that the discussion could also extend to all types of wound repair.

Skin has three layers, keratin, epidermis and dermis. If only the epidermis is damaged, as in most minor injuries, keratinocytes migrate from the edge of wound and eventually cover it, reforming the epidermis and keratin [D.R. Knighton and V.D. Fiegel, *Invest. Radiol.*, Vol 26, p. 604-611, 1991]. The risk of scar formation is thus relatively low for such minor injuries.

If all three skin layers are damaged or destroyed, new connective tissue, called granulation tissue, must first fill the wound space. This tissue is formed by deposition of ECM components by fibroblasts, which migrate into the wound space [D.R. Knighton and V.D. Fiegel, *Invest. Radiol.*, Vol 26, p. 604-611, 1991]. Although the formation of granulation tissue is clearly an important protective mechanism, the formation of such tissue can also lead to the formation of scars. Production of ECM components, such as collagen, has been particularly linked to scar formation. Scars on the skin can be both a cosmetic and a functional problem. For example, scar formation following serious burns can restrict the mobility of joints. Scar formation within other types of tissue, such as in the lungs after a bacterial infection, or in many organ tissues following surgery, can be extremely dangerous. One reason scars within organ tissues are so dangerous is that the scar does not duplicate the functionality of the original organ tissue, so that the healing of the wound does not lead to a complete restoration of organ capacity and function. Thus, clearly scar formation can be a pathological process.

One example of scarring which is both pathological and potentially clinically damaging is the formation of strictures, which is a common clinical condition, characterized by the narrowing of

a biological passageway by a noncompliant section of scar tissue. One example of such a stricture is a urethral stricture. Such scar tissue typically arises as the reaction to an insult, which may be idiopathic in origin, as a result of instrumentation and catheterization of the urethra; the result of an external trauma; or the result of urethritis, particularly when caused by a micro-organism such as *N. gonorrhea*. The obstruction of the urethra by the stricture leads to such symptoms as hesitation for urination, weaking of the urinary stream, intermittance and the feeling of incomplete urinary evacuation. In addition, such obstruction may lead to damage to the urinary bladder, ureters and kidneys.

Strictures are generally difficult to cure or even ameliorate since the most common treatment, surgical dilatation, may be accompanied by insult to the passageway and scar tissue production, which in turn could cause luminal obstruction and treatment failure. The most successful treatment, open surgery of the passageway, is complicated and requires special training. Therefore, currently available treatments are generally unable to cure strictures.

Given the etiology of strictures, particularly with regard to the appearance of such strictures after surgery or other traumas, the involvement of fibrosis caused by excessive collagen synthesis has been suggested. For example, the content of collagen type I had been found to increase, while the content of collagen type III decreased, in urethral stricture scar formation (Baskin *et al.*, *J. Urol.*, 157:371, 1997). Furthermore, biopsies taken from such strictures showed dense collagen (Singh and Scott, *Br. J. Urol.*, 47:871, 1975). Thus, clearly collagen, particular type I collagen, is an important factor in the pathology of stricture formation.

However, the deposition of ECM components, such as collagen, is currently believed to also be important for healing of the wound. Indeed, the prior art teaches that the strength of the healing wound is ultimately dependent upon collagen deposition [Haukipuro, K., *et al.*, *Ann. Surg.*, Vol. 213, p. 75-80 , 1991]. Thus, according to the prior art, collagen deposition must be present at a sufficient level to give the healing wound strength and support, yet not at such a high level to cause the formation of scars.

If collagen deposition were prevented, permanent scars might not be formed. Therefore, these pathological processes are caused, at least in part, by the synthesis of excess collagen. Furthermore, the crucial role of collagen in other clinical conditions, such as fibrosis, has prompted attempts to develop drugs that inhibit its accumulation [K.I. Kivirikko, *Annals of Medicine*, Vol. 25, pp. 113-126 (1993)].

Such drugs can act by modulating the synthesis of the procollagen polypeptide chains, or by inhibiting specific post-translational events, which will lead either to reduced formation of extra-

cellular collagen fibers or to an accumulation of fibers with altered properties. Unfortunately, only a few inhibitors of collagen synthesis and deposition are available, despite the importance of this protein in sustaining tissue integrity and its involvement in various disorders. Furthermore, many available inhibitors lack specificity for the collagen metabolic pathway. Thus, many currently available drugs have deleterious side effects.

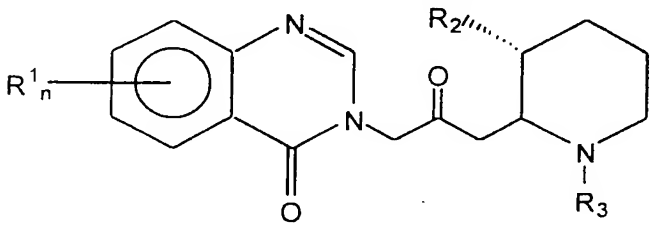
For example, cytotoxic drugs have been used in an attempt to slow the proliferation of collagen-producing fibroblasts [J.A. Casas, *et al.*, *Ann. Rhem. Dis.*, Vol. 46, p. 763 (1987)], such as colchicine, which slows collagen secretion into the extracellular matrix [D. Kershenobich, *et al.*, *N. Engl. J. Med.*, Vol. 318, p. 1709 (1988)]. Other drugs act as inhibitors of key collagen metabolism enzymes [K. Karvonen, *et al.*, *J. Biol Chem.*, Vol. 265, p. 8414 (1990); C.J. Cunliffe, *et al.*, *J. Med. Chem.*, Vol. 35, p.2652 (1992)]. However, none of these inhibitors have specific effects for the metabolism and deposition of specific types of collagen. Also, these drugs may interfere with the biosynthesis of other vital collagenous molecules, such as Clq in the classical complement pathway, acetylcholine esterase of the neuro-muscular junction endplate, conglutinin and pulmonary surfactant apoprotein. Such interference and lack of specificity could have potentially serious adverse effects.

Other drugs which can inhibit collagen synthesis, such as nifedipine and phenytoin, inhibit synthesis of other proteins as well, thereby non-specifically blocking the collagen biosynthetic pathway [T. Salo, *et al.*, *J. Oral Pathol. Med.*, Vol. 19, p. 404 (1990)]. Again, the lack of specificity significantly reduces the clinical use of these drugs, because the non-specific inhibition of protein synthesis can result in adverse side-effects when the drug is administered to the patient. Thus, simply preventing stricture formation alone does not make a compound useful if it is sufficiently non-specific to cause toxic side effects.

Indeed, clinically available anti-fibrotic drugs, including the collagen cross-linking inhibitors such as β-amino-propionitrile, are also non-specific. Unfortunately, the lack of specificity of these collagen cross-linking inhibitors ultimately results in severe side effects after prolonged use. These side effects include lathritic syndrome, as well as disrupted elastogenesis. The latter side effect is a result of the disruption of cross-linking of elastin, another fibrous connective tissue protein. In addition, the collagen cross-linking inhibitory effect of these drugs is secondary, so that collagen must first be overproduced before degradation by collagenase. Thus, a type-specific inhibitor of the synthesis of collagen itself is clearly required.

Such a type-specific collagen synthesis inhibitor was found by observing chickens which were fed extremely high levels of the coccidostat Halofuginone. These chickens were found to

have fragile, weakened skin, as evidenced by increased skin tearing, which was caused by the inhibition of collagen synthesis [Granot, I. *et al.*, *Poultry Sci.*, Vol. 70, p. 1559-1563, 1991]. Halofuginone and related compounds are disclosed in U.S. Patent No. 5,449,678 for the treatment of certain fibrotic conditions such as scleroderma and Graft Versus Host Disease. Both of these conditions are associated with excessive collagen deposition, which can be inhibited by Halofuginone. This specific inhibitor is a composition with a pharmaceutically effective amount of a pharmaceutically active compound of a formula:



wherein:

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy;

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; n is either 1 or 2; and pharmaceutically acceptable salts thereof. Of this group of compounds, Halofuginone has been found to be particularly effective for such treatment.

PCT Patent Application No. WO 96/06616 further discloses that these compounds are able to effectively treat restenosis by preventing the proliferation of vascular smooth muscle cells. Restenosis is characterized by smooth muscle cell proliferation and extracellular matrix accumulation within the lumen of affected blood vessels in response to a vascular injury [Choi *et al.*, *Arch. Surg.*, Vol. 130, p. 257-261 (1995)]. One hallmark of such smooth muscle cell proliferation is a phenotypic alteration, from the normal contractile phenotype to a synthetic one. Type I collagen has been shown to support such a phenotypic alteration, which can be blocked by Halofuginone [Choi *et al.*, *Arch. Surg.*, Vol. 130, p. 257-261 (1995); PCT Patent Application No. 96/06616]. Thus, Halofuginone can prevent such abnormal redifferentiation of smooth muscle cells after vascular injury by blocking the synthesis of type I collagen.

However, none of these studied models adequately predicts the behavior of Halofuginone in the promotion of wound healing or in the prevention of the formation of scars for a number of reasons.

First, the prior art teaches against the use of Halofuginone to promote wound healing,

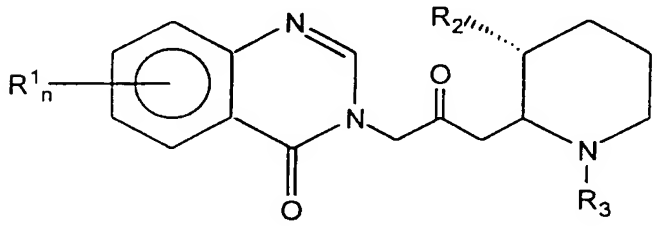
since as described above, the prior art teaches that collagen is necessary for wound healing. According to the prior art, collagen is particularly necessary for the strength of the wound. Thus, any use of Halofuginone to reduce or prevent scar formation might be expected to also prevent the healing of the wound.

Second, certainly the promotion of wound healing by Halofuginone or related compounds is neither taught nor suggested by the prior art. In fact, given the knowledge about Halofuginone and related compounds, and the expected mechanism for wound healing and scar formation, one of ordinary skill in the art would expect wound healing to be retarded in subjects receiving Halofuginone, rather than promoted. Thus, the finding that Halofuginone actually promotes wound healing without scar formation is contrary to the teachings in the art.

There is thus a widely recognized need for, and it would be highly advantageous to have, a promoter of wound healing which substantially inhibits such pathological processes as scar formation, without causing non-specific effects.

SUMMARY OF THE INVENTION

According to the present invention, there is provided a composition for promoting wound healing, comprising a pharmaceutically effective amount of a compound in combination with a pharmaceutically acceptable carrier, the compound being a member of a group having a formula:



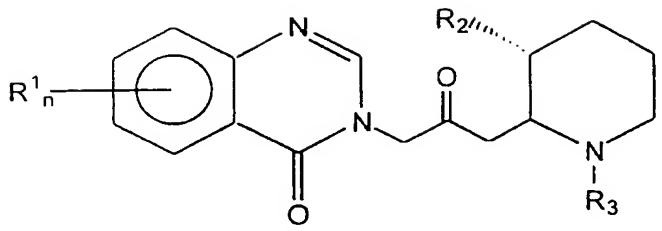
wherein:

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy, and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy; and n is either 1 or 2; and pharmaceutically acceptable salts thereof.

According to another embodiment of the present invention, there is provided a method of manufacturing a medicament for promoting wound healing, comprising the step of placing a pharmaceutically effective amount of a compound in a pharmaceutically acceptable carrier, the compound being a member of a group having a formula:



wherein:

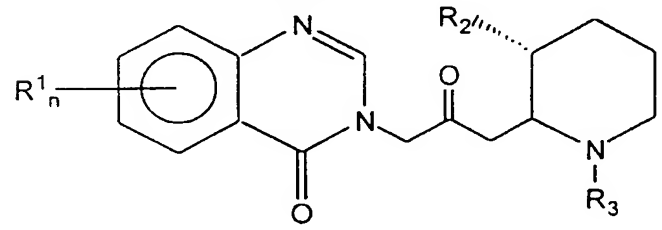
R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy, and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and *n* is either 1 or 2;

and pharmaceutically acceptable salts thereof.

According to another embodiment of the present invention, there is provided a method of manufacturing a medicament for administration before a performance of a surgical procedure, for promotion of wound healing, the method comprising the step of placing a pharmaceutically effective amount of a compound in a pharmaceutically acceptable carrier, the compound being a member of a group having a formula:



wherein:

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

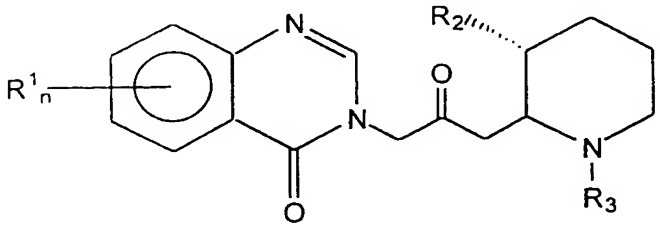
R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy, and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and *n* is either 1 or 2;

and pharmaceutically acceptable salts thereof.

According to yet another embodiment of the present invention, there is provided a composition for treatment, substantially before a performance of a surgical procedure, for

promotion of wound healing, the composition comprising a pharmaceutically effective amount of a compound having a formula:



wherein:

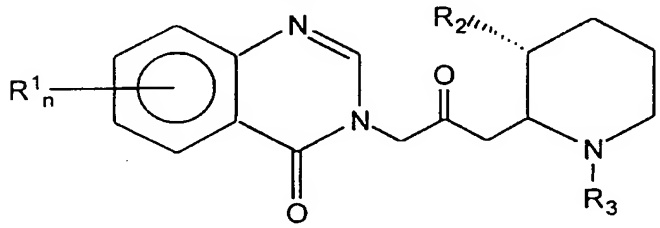
R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy, and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and n is either 1 or 2;

and pharmaceutically acceptable salts thereof.

According to still another embodiment of the present invention, there is provided a composition for treating a stricture in a subject, comprising a pharmaceutically effective amount of a compound having a formula:



wherein:

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy, and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and n is either 1 or 2;

and pharmaceutically acceptable salts thereof.

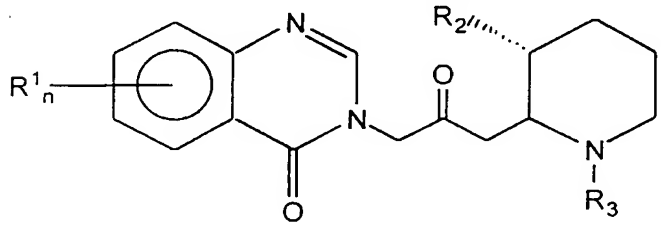
Preferably, the stricture is an urethral stricture. More preferably, the urethral stricture arises after a surgerical procedure is performed in the subject. Most preferably, the surgical procedure is catheterization of the urethra of the subject.

Alternatively and preferably, the urethral stricture arises after an infection of the urethra of

the subject.

According to preferred embodiments of the present invention, the compound is administered to the subject through transurethral administration for treatment of a urethral stricture.

According to yet another embodiment of the present invention, there is provided a method for treating a stricture in a subject, comprising the step of administering to the subject a pharmaceutically effective amount of a compound having a formula:



wherein:

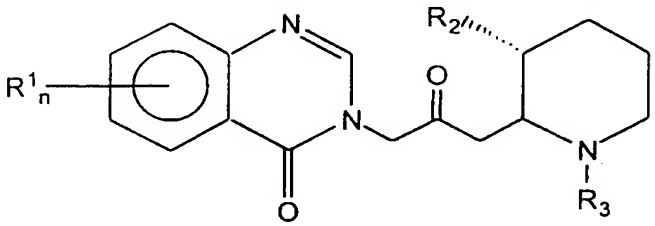
R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy, and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and n is either 1 or 2;

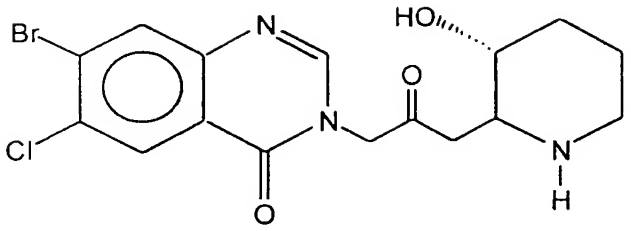
and pharmaceutically acceptable salts thereof.

According to still another embodiment of the present invention, there is provided a composition for preventing cicatrix formation in a subject while maintaining a strength of a wound, comprising a pharmaceutically effective amount of a compound having a formula:



wherein: R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy; R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy, and R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and n is either 1 or 2; and pharmaceutically acceptable salts thereof; wherein said compound is administered to the subject, such that the strength of the wound of the subject is not decreased.

For all of these embodiments, preferably the compound is Halofuginone. Hereinafter, the term "Halofuginone" is defined as a compound having a formula:



and pharmaceutically acceptable salts thereof. The composition preferably includes a pharmaceutically acceptable carrier for the compound.

Preferably, all of the compounds referred to hereinabove can be either the compound itself as described by the formula, and/or pharmaceutically acceptable salts thereof.

Hereinafter, the term "cicatrix" includes scars, strictures, hypertrophic scars, as well as substantially any other type of cicatrix. As described in greater detail below, although the examples of the efficacy of the present invention with regard to strictures are shown for urethral strictures, it is understood that this is merely an example for the sake of description and is not meant to be limiting in any way.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIGS. 1A-1H illustrate the effect of Halofuginone on wound healing;

FIG. 2 illustrates the effect of Halofuginone on wound strength;

FIG. 3 illustrates the effect of Halofuginone on the promotion of wound healing after implanting a tumor;

FIG. 4 illustrates the effect of Halofuginone on the promotion of wound healing after implanting a bladder carcinoma;

FIGS. 5A-5D illustrate the effect of Halofuginone on stricture formation in the urethra;

FIG. 6 illustrates the effect of Halofuginone on collagen $\alpha 1(I)$ gene expression in the stricture site;

FIGS. 7A-7D illustrate the effect of Halofuginone on collagen content at the stricture site;

FIGS. 8A-8C illustrate the lack of effect of Halofuginone on collagen type III at the stricture site; and

FIG. 9 illustrates the effect of Halofuginone on collagen synthesis by fibroblasts derived from rat urethra.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unexpectedly, compositions according to the present invention have been found to act as an inhibitor of pathological processes arising from wound healing, such as scar formation, while promoting wound healing itself. The present invention is also an inhibitor of the formation of strictures.

Specifically, the most preferred compound of the present invention, Halofuginone, can be used to inhibit scar formation, and promote wound healing, by preventing collagen deposition from occurring within the wound space. In examples detailed below, Halofuginone is shown to inhibit collagen deposition within the urethra following catheterization, thereby inhibiting the formation of urethral strictures.

In other examples given below, Halofuginone is shown to not interfere with wound healing. Such an effect is particularly unexpected because Halofuginone decreases collagen deposition. However, collagen deposition is required to strengthen the healing wound. Furthermore, high levels of Halofuginone lead to decreased skin strength and increased skin tearing. Based upon the prior art, Halofuginone would be expected to obstruct wound healing. Yet, contrary to the teachings of the prior art, Halofuginone has been specifically shown to promote wound healing, an effect which is both novel and non-obvious.

Based upon these novel, non-obvious and completely unexpected results, Halofuginone could clearly be used in a number of ways for the promotion of wound healing, as well as to maintain wound strength during the process of wound healing. For example, Halofuginone could be used to either treat formed strictures and other scars, which can be generally described as a cicatrix, such as those following surgery or inflammatory disease, or to substantially inhibit the formation of those strictures or other scars, or other examples of a cicatrix.

Halofuginone can also be used as a pretreatment, administered to a subject before surgery to substantially prevent the formation of a cicatrix. Of course, such a pretreatment would be most effective for scheduled surgery, as that would allow Halofuginone to be administered for a sufficient period of time before surgery to be most effective.

The present invention may be more readily understood with reference to the following illustrative examples and figures. It should be noted that although reference is made exclusively to Halofuginone, it is believed that the other quinazolinone derivatives described and claimed in U.S. Patent 3,320,124, the teachings of which are incorporated herein by reference, have similar properties.

Example 1

Effect of Halofuginone on Wound Healing

The effect of Halofuginone on wound healing was examined by using mice which were first irradiated and then wounded. As shown in Figure 1, although Halofuginone treatment caused a reduction in the collagen content of the wounded and irradiated skin, the wound still healed.

The experiment was conducted as follows. First, C3H, defined-flora, pathogen-free female mice of 12-14 weeks of age were anesthetized with 60 mg/kg sodium phenobarbital. The mice were then shaved. One group of mice was then irradiated as follows. First, a flap of skin about 40 mm long and about 20 mm wide was pulled through a slit in the lead cover of an irradiation jig and secured with tape, so that only the flap of skin was exposed. This exposed skin was then irradiated by using a 175 kVp/20 mA orthovoltage X-ray source, with a 2 mm Cu filter at a dose rate of 1.0 Gy/min. A standard dose of 18 Gy was delivered.

All of the mice were then wounded by making a full depth incision, about 25 mm long, in the skin along the midline of the lower back. Note that for the irradiated animals, the wound was made within the irradiated area, immediately following irradiation. For all mice, the incision was immediately closed by 3-4 metallic wound closure clips which were removed 2 days later.

After wounding, the mice were injected i.p. every other day, either with 1 mg per mouse of Halofuginone or with saline as a control. After 14 days, 2 days following the last injection, the mice were sacrificed and skin samples were collected into phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde in PBS at 4 °C. Serial 5 μm sections were prepared after the samples had been dehydrated in graded ethanol solutions, cleared in chloroform and embedded in Parafin. The sections were deparafinized in xylene, rehydrated through a graded series of ethanol solutions, rinsed in distilled water and treated with 0.125 mg/ml pronase in 50 mM Tris-HCl, 5 mM EDTA, pH 7.5 for 10 minutes. After digestion, slides were rinsed in distilled water, postfixed in 10% formalin in PBS, blocked in 0.2% glycine, rinsed in distilled water again, rapidly dehydrated through a graded ethanol solution and air-dried for several hours. Samples were then stained with hematoxylin-eosin (Figures 1A-D). Immunohistochemistry was performed with specific rabbit immune serum to rat collagen type I (Laboratoire de Pathologie Cellulaire. Institut Pasteur de Lyon, Lyon, France) and secondary rat anti-rabbit FITC-conjugated McAb (Figures 1E-H).

Figures 1A and 1E show tissue taken from the wound of a mouse treated with saline. Figures 1B and 1F show tissue taken from the wound of a mouse treated with Halofuginone.

Figures 1C and 1G show tissue taken from the wound of an irradiated mouse treated with saline. Figures 1D and 1H show tissue taken from the wound of an irradiated mouse treated with Halofuginone. Essentially, Figure 1G shows that collagen content was higher in the wound of an irradiated mouse. However, Figure 1H shows that collagen content was significantly lowered by treatment with Halofuginone. Yet, all of these wounds healed, regardless of the collagen content.

Wound strength was assessed by preparing rats substantially as described above, except that one group of rats (not irradiated) only received one injection of Halofuginone after wounding. The strength of wounds was measured as follows. First, a square of skin which was approximately 20 mm long and 16 mm wide, and which included the main part of the wound, was excised from sacrificed rats. Next, the skin was cut, perpendicular to the wound, to yield 7 strips of skin, each of which was 2 mm wide. The skin strips were secured between paper reinforcement frames and loaded on an Instron tensiometer for stretching at a constant rate of 25 mm/min. The bursting point was then recorded. Data are shown in Figure 2 for non-irradiated animals which did not receive Halofuginone (column 1), non-irradiated animals which received one (column 2) or six (column 3) injections of Halofuginone, irradiated animals which did not receive Halofuginone (column 4) and irradiated animals which received six injections of Halofuginone (column 5).

Figure 2 clearly demonstrates that Halofuginone did not reduce wound strength, whether animals were irradiated or not. However, as noted above, the prior art teaches the importance of collagen for wound strength, so that Halofuginone would be expected to reduce such wound strength. Thus, the results obtained in Figures 1 and 2 are clearly novel and non-obvious, as well as teaching against the prior art, since Halofuginone does not reduce wound strength.

Example 2

Halofuginone Improves Wound Healing in Mice with Tumors

Tumors from C6 rat glioma cells were prepared and implanted in nude mice. Certain mice received Halofuginone, either orally or through i.p. (intra-peritoneal) administration. The results showed that in mice which received Halofuginone, wound healing was promoted substantially without scar formation. The experimental method was as follows.

C6 rat glioma cells were cultured in DMEM supplemented with 5% FCS, 50 units/ml penicillin, 50 micrograms/ml streptomycin and 125 micrograms/ml fungizone. Aggregation of

cells into small spheroids of about 150 microns was initiated by replating cells from confluent cultures onto agar-coated bacteriological plates. After 4-5 days in culture, the suspension was transferred to a 250 ml spinner flask (Bellco, USA), and the medium was changed every other day for 30-40 days. All culture operations were performed at 37 °C and 5% CO_2. Other conditions were as previously reported (Abramovitch *et al.*, *Br. J. Cancer*, 77:440-447, 1998; Abramovitch *et al.*, *Cancer Res.*, 55:1956-62, 1995).

For the implantation of the cells, male CD1-nude mice, two months old and 30 g body weight, were anesthetized. A single tumor, about 1 mm in diameter, was implanted in each mouse subcutaneously in the lower back at the site of a 4 mm incision, using a Teflon tubing, as reported previously (Abramovitch *et al.*, *Br. J. Cancer*, 77:440-447, 1998). The incision was formed with fine surgical scissors and closed with an adhesive bandage.

The mice were then divided into six groups. One group received Halofuginone by oral administration as previously described. Four groups received different concentrations of Halofuginone through i.p. injections every other day (0.1, 0.5, 2 and 4 micrograms of Halofuginone per injection). One group received sham injections (no Halofuginone).

MRI microimaging of the implanted tumor was performed on a horizontal 4.7 T Bruker-Biospec spectrometer using an actively RF decoupled surface coil, 2 cm in diameter, and a bird-cage transmission coil, as reported previously (Abramovitch *et al.*, *Br. J. Cancer*, 77:440-447, 1998; and Abramovitch *et al.*, *Magn. Reson. Med.*, 39:813-824, 1998). Mice were anesthetized and placed supine with the tumor located at the center of the surface coil. Gradient echo images were acquired with a slice thickness of 0.5 mm, TE of 10 ms, TR of 230 ms and 256x256 pixels matrix resulting in in-plane resolution of 110 microns. Growth of the capillary bed was reflected by reduction of the mean intensity at a region of interest of 1 mm surrounding the tumor (Abramovitch *et al.*, *Br. J. Cancer*, 77:440-447, 1998; and Abramovitch *et al.*, *Magn. Reson. Med.*, 39:813-824, 1998).

At the end of the experimental period, the mice were sacrificed, and sections were obtained and prepared as previously described above.

The results are shown in Figure 3. Column 1 is an external photograph of the tumor, column 2 is a photograph of the incision site, column 3 is an MRI, column 4 is collagen $\alpha 1(I)$ gene expression, column 5 shows the collagen content and column 6 is the histology of the incision. From these results, Halofuginone significantly improved wound healing in mice, whether administered orally or as 4 micrograms per i.p. injection, despite the presence of the tumor (Figure 3), such that no scarring is found at the site of the wound. Such a result is

surprising since wounds located less than 1 mm from a tumor implantation site have been previously shown not to heal rapidly. For example, re-epitheliazation was not completed in such wounds even three weeks after the incision was made, and the wound site was found to still feature inflammatory cells and blood clots (Abramovitch *et al.*, *Br. J. Cancer*, 77:440-447, 1998). By contrast, histological sections of the tumors demonstrated that Halofuginone induced complete re-epitheliazation in 4 out of 5 mice in each group, whether administered orally or as 4 micrograms per i.p. injection, while for all other groups, re-epitheliazation occurred in only 1 out of 5 mice in each group.

Columns 4 and 5 show that both the expression of the collagen $\alpha 1(I)$ gene and the collagen content was decreased in rats which received Halofuginone, particularly at the highest dose (4 micrograms per i.p. injection). Thus, the decreased collagen content in mice which received Halofuginone is surprisingly accompanied by increased re-epitheliazation of the wound and the promotion of wound healing.

Similar visual results were obtained with mice implanted with T50 bladder carcinoma tumors (Figure 4). The experimental results were as follows. C3H mice were divided into two groups of 6 mice each. The experimental group received a diet containing either 10 mg/kg or 5 mg/kg of Halofuginone 3 days prior to the injection of T50 bladder carcinoma cells and during 2 weeks after. Cultured T50 cells, a more aggressive variant of the chemically induced MBT2 mouse bladder carcinoma, were dissociated with trypsin/EDTA into a single cell suspension (10^6 cells/ml) in growth medium and inoculated s.c. in two sites on the dorsa of mice. The right side received $0.4 * 10^5$ cells, and the left side received $2 * 10^5$ cells. The experiment ended at day 17.

Figure 4 is a photograph of representative bladder carcinoma bearing mice which were untreated (top) or treated (bottom) with Halofuginone. Clearly, mice which were treated with Halofuginone had greater wound healing than mice which did not receive Halofuginone. Halofuginone was clearly able to promote wound healing in mice implanted with bladder carcinoma tumors. Thus, the decreased collagen content in mice which received Halofuginone is surprisingly accompanied by increased re-epitheliazation of the wound and the promotion of wound healing.

Example 3

Effect of Halofuginone on Stricture Formation

The effect of Halofuginone in post-trauma stricture formation in rats was studied. Briefly, urethral strictures were induced by catheterization and application of an electrical current to the

urethra of rats. The rats were divided into four groups: untreated rats as controls (Figure 5A); rats treated with Halofuginone but not with coagulation current (Figure 5B); rats treated with coagulation current alone (Figure 5C); and rats treated with both Halofuginone and coagulation current (Figures 5D-5F). Rats which received only coagulation current had clear strictures, while rats treated with both Halofuginone and coagulation current did not have such strictures.

The experiments were performed as follows. First, urethral catherization was performed on anesthetized rats with a 23G Quik-Cath (Baxter, Ireland) sheath. A spinal needle was inserted into the sheath, and was used to apply a coagulation current at a level of 10 W to the outer end of the needle for 1 second at three locations, 8, 10 and 12 mm from the meatus, in order to produce urethral strictures. Halofuginone was given for 7 days starting at the day of stricture formation, either orally at concentrations of 1 ppm and 5 ppm in the diet, or by injection of 0.03% Halofuginone dissolved in 2% lignocaine directly into the urethra once a day. Urethral gross morphology was evaluated by urethrogram after injecting contrast material (Telebrix Megalumine 300 mg/ml, Laboratorie, Guerbet, Aulnay Sous Bios, France) into the anesthetized rat through a 23G Quik-Cath sheath, under fluoroscopy 3 weeks after stricture formation.

As shown in Figure 5C, rats treated with coagulation current alone displayed reproducible urethral strictures, accompanied by ballooning of the urethra proximal to the narrowed urethra. Also, as shown in Figure 5A, control, untreated rats, which received neither coagulation current nor Halofuginone, did not have any strictures. Furthermore, rats which received local Halofuginone with lignocaine alone, without coagulation current, also did not have any strictures. as shown in Figure 5B.

By contrast, rats which received both locally administered Halofuginone and coagulation treatment did not have strictures, nor did rats which received 5 ppm of Halofuginone in the diet with coagulation treatment (Figures 5E and 5F). The administration of only 1 ppm of Halofuginone in the diet did not affect stricture formation (Figure 5D). Thus, the administration of sufficient Halofuginone orally for 7 days after strictures were induced, or local administration of Halofuginone, was able to prevent the formation of strictures after coagulation treatment.

Example 4

Involvement of Collagen in Urethral Stricture Formation and the Effect of Halofuginone

The involvement of collagen, as well as the effect of Halofuginone, in post-trauma urethral stricture formation in rats was studied. Briefly, collagen-specific staining techniques, as

well as hybridization with a labeled collagen-specific genetic probe, demonstrated the importance of collagen as a component of urethral strictures, as shown in Figures 6 and 7A-7D.

The experiments were performed as follows. First, urethral catherization was performed on the rats with a 23G Quik-Cath (Baxter, Ireland) sheath. A spinal needle was inserted into the sheath, and was used to apply a coagulation current at a level of 10 W to the outer end of the needle for 1 second at three locations, 8, 10 and 12 mm from the meatus, in order to produce urethral strictures. Halofuginone was given for 7 days starting at the day of stricture formation, either orally at concentrations of 1 ppm and 5 ppm in the diet, or by injection of 0.03% Halofuginone dissolved in 2% lignocaine directly into the urethra once a day. After 21 days, the rats were sacrificed and the sutures were reopened to determine the level of urethral stricture formation, at which time biopsies of the tissue were taken.

The tissue was sectioned so that histological studies could be performed. Briefly, the tissue samples were collected into phosphate-buffered saline (PBS) and fixed overnight in 4% paraformaldehyde in PBS at 4 °C. Serial 5 μm sections were prepared after the samples had been dehydrated in graded ethanol solutions, cleared in chloroform and embedded in Paraplast. Differential staining of collagenous and non-collagenous proteins was performed with 0.1% Sirius red and 0.1% fast green as a counter-stain in picric acid. This procedure stains collagen red [Gascon-Barre, M., *et al.*, *J. Histochem. Cytochem.*, Vol 37, p. 377-381, 1989]. The results are shown in Figures 7A-7D.

For hybridization with the genetic probe, the sections were deparafinized in xylene, rehydrated through a graded series of ethanol solutions, rinsed in distilled water for 5 minutes and then incubated in 2X SSC at 70 °C for 30 minutes. The sections were then rinsed in distilled water and treated with pronase, 0.125 mg/ml in 50 mM Tris-HCl, 5 mM EDTA, pH 7.5, for 10 minutes. After digestion, the slides were rinsed with distilled water, post-fixed in 10% formalin in PBS and blocked in 0.2% glycine. After blocking, the slides were rinsed in distilled water, rapidly dehydrated through graded ethanol solutions and air-dried for several hours. The sections were then hybridized with a genetic probe.

Before hybridization, the genetic probe was prepared by cutting out the 1600 bp rat collagen α1(I) insert from the original plasmid, pUC18. The 1600 bp insert was then inserted into the pSafyre plasmid. The sections were then hybridized with this probe after digoxigenin-labelling. Alkaline phosphatase activity was detected in the sections as previously described [Knopov, V., *et al.*, *Bone*, Vol 16, p. 329S-334S, 1995]. The results are shown in Figure 6.

Figure 6 shows that the cells in the biopsies taken from the urethral strictures are

specifically expressing the collagen α1(I) gene, indicating the presence of collagen type I. Note that each brown dot represents a cell expressing the collagen α1(I) gene. By contrast, tissue taken from rats which received Halofuginone orally, as well as rats which did not receive coagulation treatment, had significantly reduced levels of expression of the collagen α1(I) gene. Indeed, image analysis of the results (3 biopsies for each type of treatment) demonstrated a 6.8 fold increase in the expression of the collagen α1(I) gene after treatment with coagulation treatment. By contrast, Halofuginone treatment resulted in a 5.3 fold reduction in the expression of the collagen α1(I) gene, which is an almost complete abolition of the increased expression. Thus, clearly the stricture tissue from rats which received the coagulation current alone expressed high levels of the collagen α1(I) gene, while rats which received Halofuginone and coagulation current had almost normal levels of expression.

The increased expression of the collagen α1(I) gene was accompanied by increased collagen content of the tissue, as shown in Figure 7. Figure 7B shows a section of tissue taken from one of the strictures and stained with Sirius red, while Figure 7A shows such a section of tissue taken from a control, non-treated rat. Clearly, much higher levels of staining by Sirius red are found in Figure 7B, indicating the presence of collagen in the stricture tissue. Halofuginone treatment, whether given orally (Figure 7C) or locally (Figure 7D), decreased the amount of collagen in the biopsies and prevented the lumen from filling with collagen. Thus, clearly high levels of collagen type I are present in the lumen of urethral strictures which are induced in the rats, while such high levels are abolished by the administration of Halofuginone.

Example 5
Halofuginone Does Not Alter Levels of Collagen Type III

Immunohistochemistry with antibodies to collagen type III was performed on biopsies taken from control, untreated rats, rats treated with coagulation current alone and rats fed a diet containing Halofuginone after coagulation current treatment. No significant changes were observed in the levels of collagen type III between the different groups of rats (Figure 8). The experimental method was as follows.

Rats were treated and sections of biopsy tissue were prepared as described previously in Example 4. Immunohistochemistry was performed with primary antibodies against collagen type III (BioGenex, San Ramon, California, USA) and a Histomouse SP kit (Zymed Laboratories Inc., South San Francisco, California, USA) for the secondary antibodies. The primary

antibodies were used in a 1:1000 dilution and detection was performed with the Histomouse SP kit according to the instructions of the manufacturer.

Figure 8A shows immunohistochemistry on biopsies taken from control, untreated rats; Figure 8B shows immunohistochemistry on rats treated with coagulation current alone; and Figure 8C shows immunohistochemistry on rats fed a diet containing Halofuginone after coagulation current treatment. No significant changes were observed in the levels of collagen type III between the different groups of rats. Thus, clearly the levels of collagen type III were not changed by the induction of urethral strictures or by the administration of Halofuginone.

Example 6

Effect of Halofuginone on Fibroblasts from Urethra

The effect of Halofuginone on fibroblasts obtained from normal rat urethra was examined. The fibroblasts were cultured from sections of urethral tissue, and then incubated in the presence or absence of Halofuginone. As shown in Figure 9, even low concentrations of Halofuginone decreased the level of collagenase-digestible proteins (CDP), while not affecting the level of non-collagenase digestible proteins (NCDP). The decreased level of CDP was shown to be caused by decreased expression of the collagen $\alpha 1(I)$ gene. The experimental method was as follows.

Urethral fibroblasts were obtained from three normal male rats, and were processed under sterile conditions. The urethra was cut into 1-2 mm pieces and rinsed several times in a solution containing 0.2 ml/10 ml penicillin/streptomycin and 50 micrograms/ml gentamycin. Each piece of urethra was placed in a 1 ml well of a 24 well plate (Greiner Labortchnik, Germany). Each well was filled with DMEM (Dulbecco's Modified Eagle's Medium), containing 4.5 g/ml of D-glucose, 25% FCS (fetal calf serum), 0.1 ml/10 ml penicillin/streptomycin, 0.1 ml/10 ml glutamine, 50 micrograms/ml gentamycin and 2.5 micrograms/ml amphotericin B. The wells were incubated in an incubator containing 5% CO_2 at 37 °C. Half of the medium was replaced after 10 days. Two to three days later the cultures were trypsinized with trypsin-EDTA medium. The content of each pair of wells was combined, resuspended in DMEM and placed into one 250-ml flask containing 5 ml of medium. When the cell cultures reached confluency, they were transferred to new flasks with a 1:5 ratio of old to new medium. The concentration of FCS was then decreased to 10%.

For the evaluation of the synthesis of CDP and NCDP, cells were incubated for 24 hours

with various concentrations of Halofuginone in 0.5 m glutamine-free DMEM containing 5% FCS, ascorbic acid (50 micrograms/ml), β-aminopropionitrile (50 micrograms/ml) and 2 micro-curies of [^{3}H]proline. At the end of the incubation period, the medium was decanted and incubated with or without collagenase for 18 hours, followed by TCA precipitation. The amount of radiolabelled collagen was estimated as the difference between total [^{3}H]proline containing proteins and protein remaining after collagenase digestion. The results are shown in Figure 9.

Next, total RNA was isolated using the guanidinium-thiocyanate-phenol technique (Chomczynski, P. and N. Sacchi, *Anal. Biochem.*, **162**:156, 1987). RNA was subjected to 1% agarose denaturing gel electrophoresis, followed by blotting onto a nylon filter (GeneScreen Plus, New England Nuclear, Boston, Massachusetts, USA). The probe for the collagen α1(I) gene was labeled using the random primer procedure with a commercial kit (Boehringer, Germany). Hybridization was performed overnight at 40 °C in a solution containing 10% dextran sulfate, 1% SDS, 1 M NaCl, 40% formamide and 200 mg/ml denatured herring sperm DNA. Hybridization was followed by two 30-minute washes in 2x SSC (1x SSC contains 0.15 M NaCl and 0.015 M sodium citrate), 1% SDS, and two 20 minute washes in 1 x SSC, 0.1% SDS. The filters were exposed to X-ray film (Agfa-Curix) at -70 °C, using intensifying screens.

From the results shown in Figure 9, Halofuginone inhibited the levels of CDP by 40% even at concentrations as low as 10^{-8} M Halofuginone. The levels of proteins were determined from the amount of protein exported into the medium by the cultured fibroblasts, of which 52% were CDP, while the remainder were NCDP by definition. The inhibitory effect of Halofuginone did not affect the appearance of NCDP or the number of cells. The specific inhibitory effect of Halofuginone for CDP was shown to be the result of a decrease in the expression of the collagen α1(I) gene, as demonstrated by Northern blot analysis. Thus, Halofuginone specifically decreased the expression of the collagen α1(I) gene in fibroblasts taken from urethral tissue, thereby decreasing the amount of CDP while not affecting NCDP.

Example 7

Suitable Formulations for Administration of Halofuginone

Halofuginone can be administered to a subject in a number of ways, which are well known in the art. Hereinafter, the term "subject" refers to the human or lower animal to whom Halofuginone was administered. For example, administration may be done topically (including opthalmically, vaginally, rectally, intranasally), orally, or parenterally, for example by intravenous

drip or intraperitoneal, subcutaneous, or intramuscular injection. A particularly preferred route of administration for the treatment or prevention of urethral strictures is transurethral administration.

Formulations for topical administration may include but are not limited to lotions, ointments, gels, creams, suppositories, drops, liquids, sprays and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. In addition, topical administration may be aided with bandages soaked in, or otherwise containing, media with the compound. The bandages can be occlusive or non-occlusive. Furthermore, a special device can be used to apply the compound. The device of the present invention includes a composition with the compound and a pharmaceutically acceptable carrier, and a container for containing the composition. Preferably, the container is a substantially sealed, sterile container, such as an aerosol-dispersing pump or a spray can. Alternatively and preferably, the container is a substantially sterile bandage. Also alternatively and preferably, the container is a squeezable tube or a gel-dispersing pump. One of ordinary skill in the art could easily select suitable containers for the composition depending upon the properties of the preferred carrier.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, sachets, capsules or tablets. Thickeners, diluents, flavorings, dispersing aids, emulsifiers or binders may be desirable.

Formulations for parenteral administration may include but are not limited to sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

Formulations for transurethral administration may include but are not limited to suspensions or solutions in water or non-aqueous media, optionally with a buffering compound added to the media.

Dosing is dependent on the severity of the symptoms and on the responsiveness of the subject to Halofuginone. Persons of ordinary skill in the art can easily determine optimum dosages, dosing methodologies and repetition rates.

Example 8

Methods of Treatment of Scars and Promotion of Wound Healing

As noted above, Halofuginone has been shown to be an effective promotor of wound healing and inhibitor of scar formation, including strictures, as well as a general inhibitor of different types of cicatrix. The following examples are illustrations only of methods of treating and preventing the formation of cicatrix such as scars, including urethral strictures, and promoting

wound healing with Halofuginone, and are not intended to be limiting.

The method includes the step of administering Halofuginone, in a pharmaceutically acceptable carrier as described in Example 7 above, to a subject to be treated. Halofuginone is administered according to an effective dosing methodology, preferably until a predefined endpoint is reached, such as the absence of clinical symptoms in the subject. For example, if a subject already had a wound, the endpoint could be the reduction in size of the wound or complete healing of the wound.

Halofuginone can also be used as a pretreatment, administered to a subject before surgery to substantially prevent the formation of cicatrix such as scars and strictures, as well as promote wound healing. Of course, such a pretreatment would be most effective for scheduled surgery, as that would allow Halofuginone to be administered for a sufficient period of time before surgery to be most effective. Halofuginone would be particularly useful for cosmetic surgery, in which the inhibition of scar formation is particularly important.

Hereinafter, the term "treatment" includes both pretreatment, before a pathological condition has arisen, and treatment after the condition has arisen. For example, treatment of a wound would include both administration of Halofuginone both before and after the genesis of the wound. The term "treating" includes both treating the subject after the pathological condition has arisen, and preventing the development of the pathological condition.

Example 9

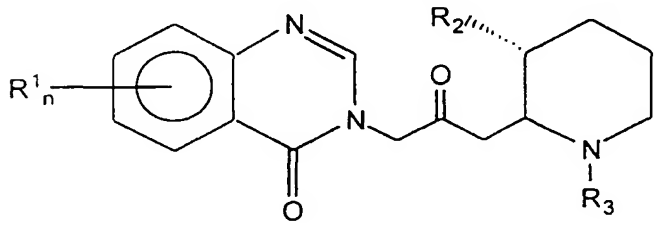
Method of Manufacture of a Medicament Containing Halofuginone

The following is an example of a method of manufacturing Halofuginone. First, Halofuginone is synthesized in accordance with good pharmaceutical manufacturing practice. Examples of methods of synthesizing Halofuginone, and related quinazolinone derivatives, are given in U.S. Patent No. 3,338,909. Next, Halofuginone is placed in a suitable pharmaceutical carrier, as described in Example 7 above, again in accordance with good pharmaceutical manufacturing practice.

While the invention has been described with respect to a limited number of embodiments, it will be appreciated that many variations, modifications and other applications of the invention may be made.

WHAT IS CLAIMED IS:

1. A composition for promoting wound healing, comprising a pharmaceutically effective amount of a compound in combination with a pharmaceutically acceptable carrier, said compound being a member of a group having a formula:



wherein:

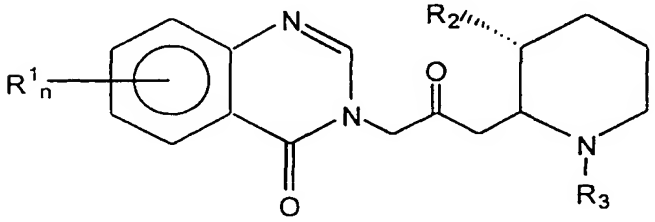
R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy, and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy; and n is either 1 or 2; and pharmaceutically acceptable salts thereof.

2. A composition according to claim 1, wherein said compound is Halofuginone and pharmaceutically acceptable salts thereof.

3. A method of manufacturing a medicament for promoting wound healing, comprising the step of placing a pharmaceutically effective amount of a compound in a pharmaceutically acceptable carrier, said compound being a member of a group having a formula:



wherein:

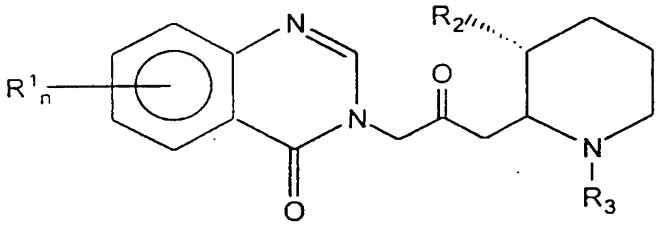
R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy, and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and *n* is either 1 or 2;

and pharmaceutically acceptable salts thereof.

4. A method of manufacturing a medicament for administration before a performance of a surgical procedure, for promotion of wound healing, the method comprising the step of placing a pharmaceutically effective amount of a compound in a pharmaceutically acceptable carrier, said compound being a member of a group having a formula:



wherein:

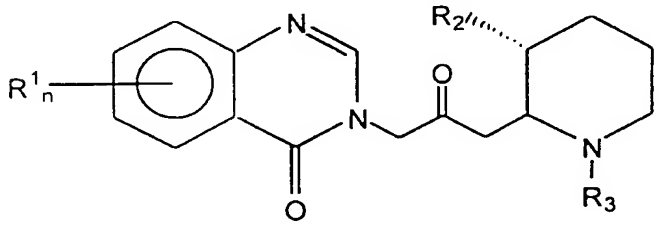
R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy, and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and *n* is either 1 or 2;

and pharmaceutically acceptable salts thereof.

5. A composition for treatment, substantially before a performance of a surgical procedure, for promotion of wound healing, the composition comprising a pharmaceutically effective amount of a compound having a formula:



wherein:

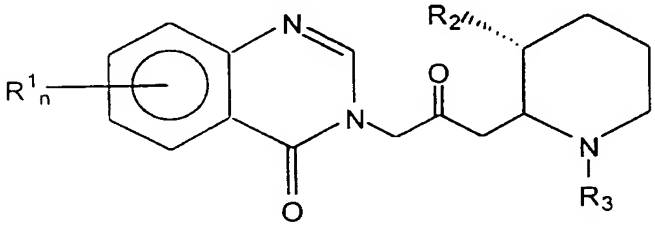
R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy, and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and *n* is either 1 or 2;

and pharmaceutically acceptable salts thereof.

6. A composition for treating a stricture in a subject, comprising a pharmaceutically effective amount of a compound having a formula:



wherein:

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy, and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and *n* is either 1 or 2;

and pharmaceutically acceptable salts thereof.

7. The composition of claim 6, wherein the stricture is an urethral stricture.

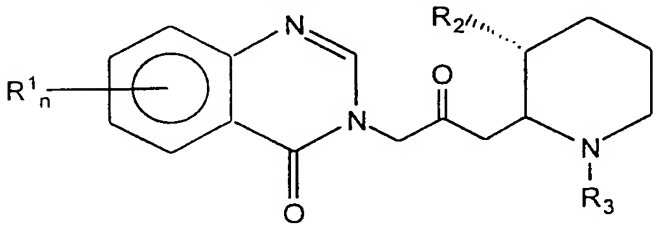
8. The composition of claim 7, wherein the urethral stricture arises after a surgerical procedure is performed in the subject.

9. The composition of claim 8, wherein said surgical procedure is catheterization of the urethra of the subject.

10. The composition of claim 7, wherein the urethral stricture arises after an infection of the urethra of the subject.

11. The composition of claim 7, wherein said compound is administered to the subject through transurethral administration.

12. A method for treating a stricture in a subject, comprising the step of administering to the subject a pharmaceutically effective amount of a compound having a formula:



wherein:

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy, and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and *n* is either 1 or 2;

and pharmaceutically acceptable salts thereof.

13. The method of claim 12, wherein the stricture is an urethral stricture.

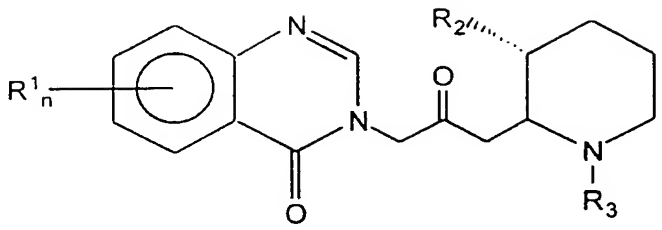
14. The method of claim 13, wherein the urethral stricture arises after a surgerical procedure is performed in the subject.

15. The method of claim 14, wherein said surgical procedure is catheterization of the urethra of the subject.

16. The method of claim 13, wherein the urethral stricture arises after an infection of the urethra of the subject.

17. The method of claim 13, wherein said compound is administered to the subject through transurethral administration.

18. A composition for preventing cicatrix formation in a subject while maintaining a strength of a wound. comprising a pharmaceutically effective amount of a compound having a formula:



wherein:

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy, and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl; and *n* is either 1 or 2;

and pharmaceutically acceptable salts thereof;

wherein said compound is administered to the subject, such that the strength of the wound of the subject is not decreased.

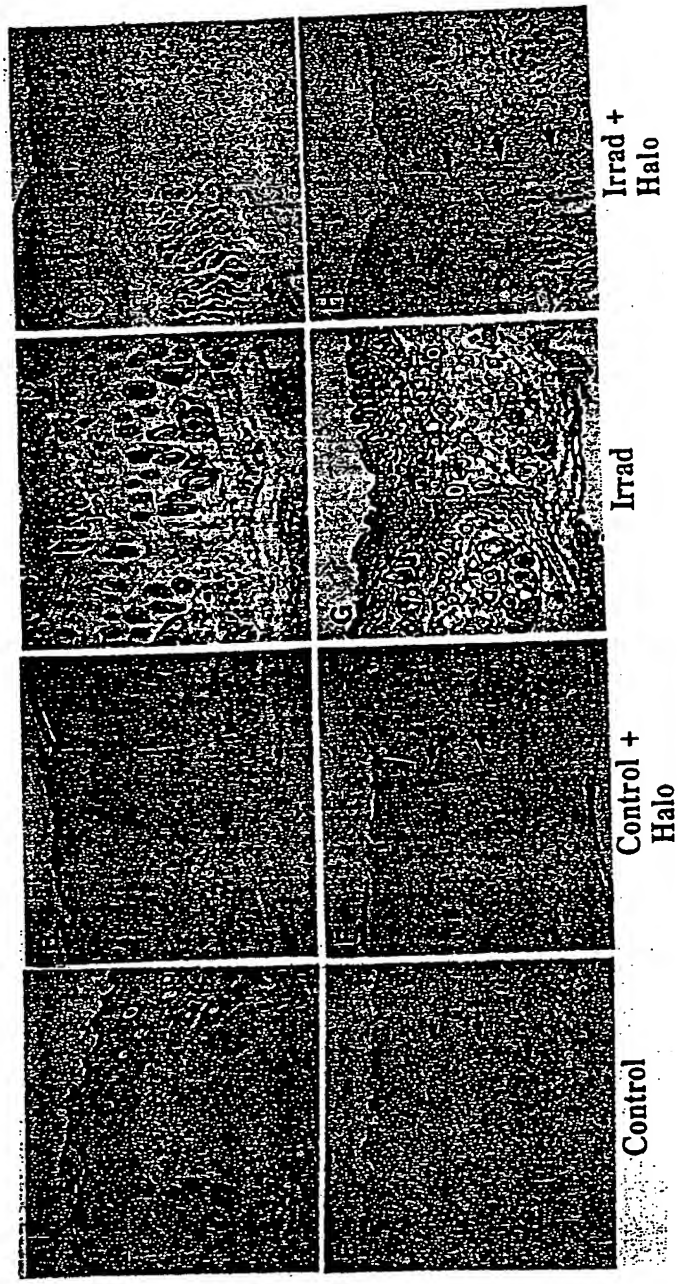


FIG. 1D
FIG. 1C
FIG. 1B
FIG. 1A
Irrad + Halo
Irrad
Control + Halo
Control
FIG. 1H
FIG. 1G
FIG. 1F
FIG. 1E

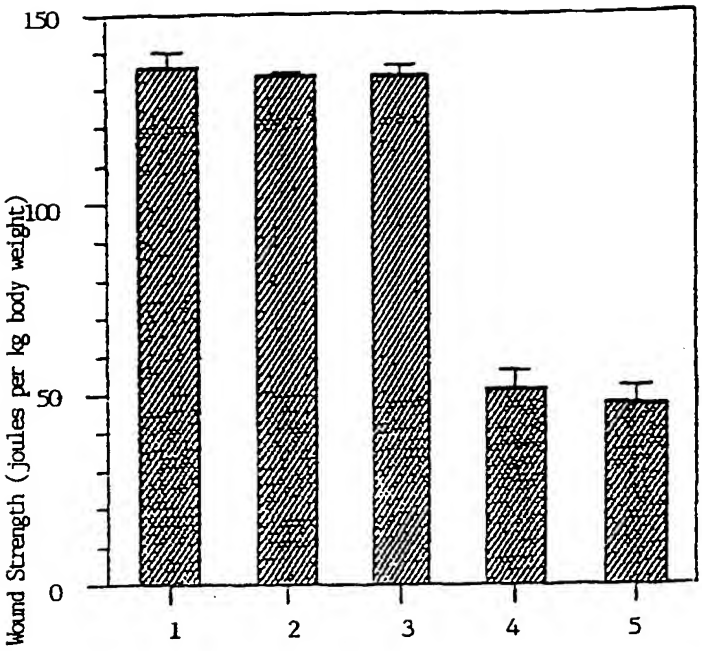


FIG. 2

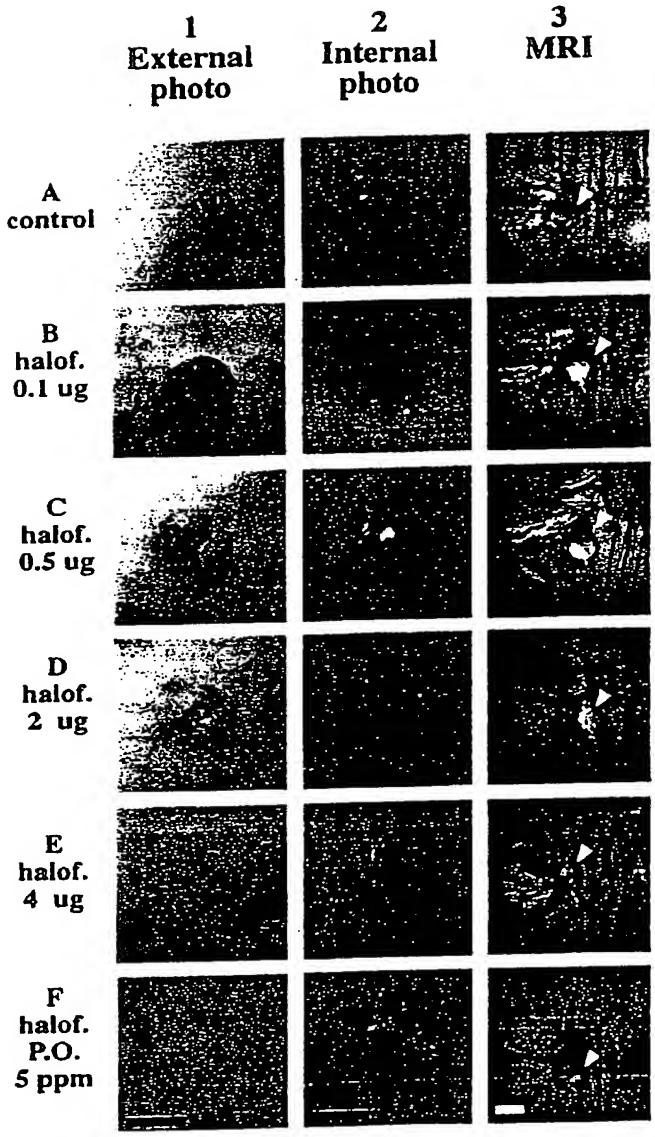

1
External
photo
2
Internal
photo
3
MRI
A
control
B
halof.
0.1 ug
C
halof.
0.5 ug
D
halof.
2 ug
E
halof.
4 ug
F
halof.
P.O.
5 ppm

FIG. 3

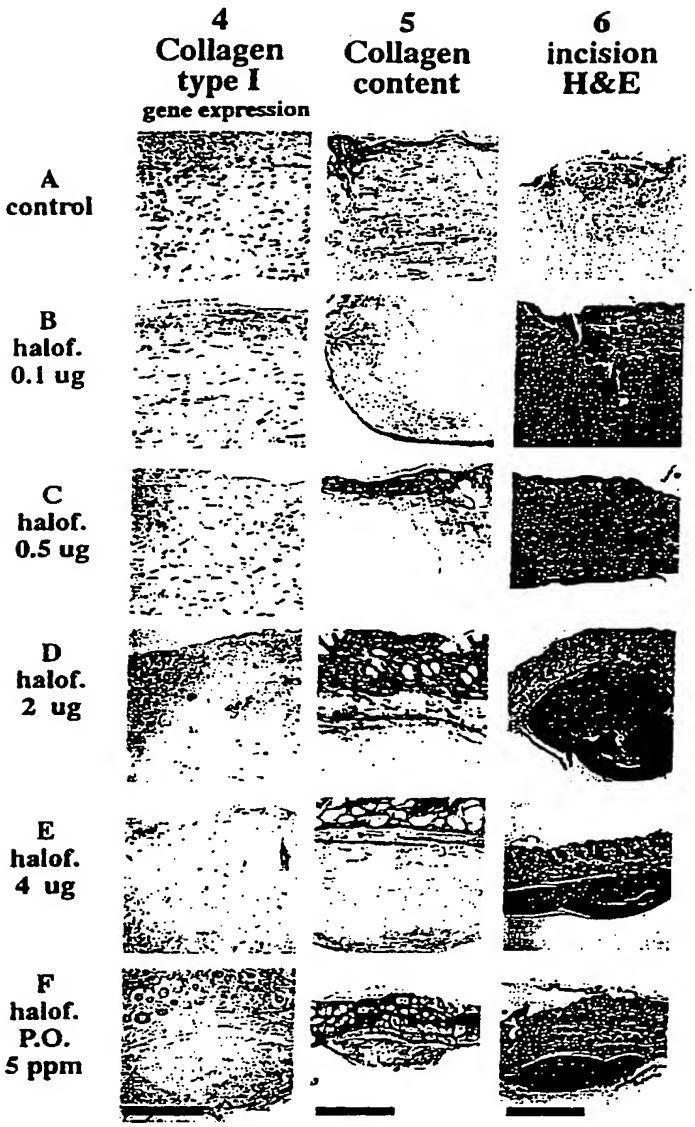


FIG. 3 (continued)

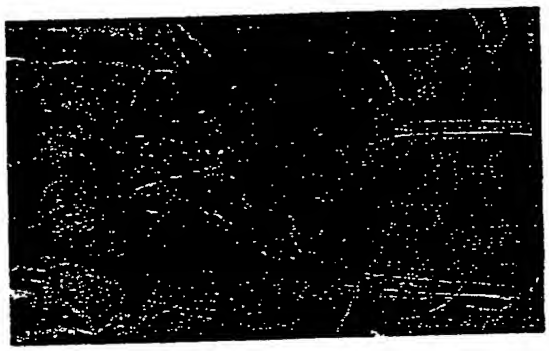


FIG. 4

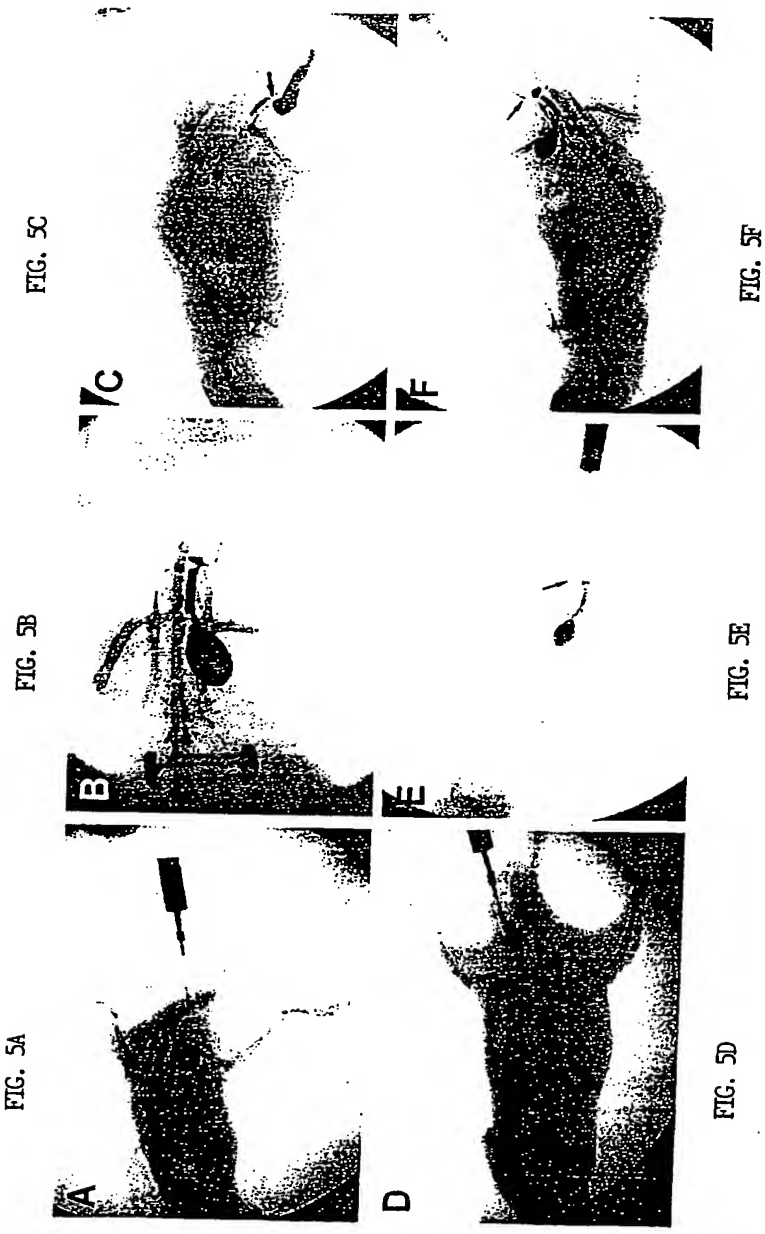


FIG. 5C
FIG. 5F
FIG. 5B
FIG. 5E
FIG. 5A
FIG. 5D
C
F
B
E
A
D

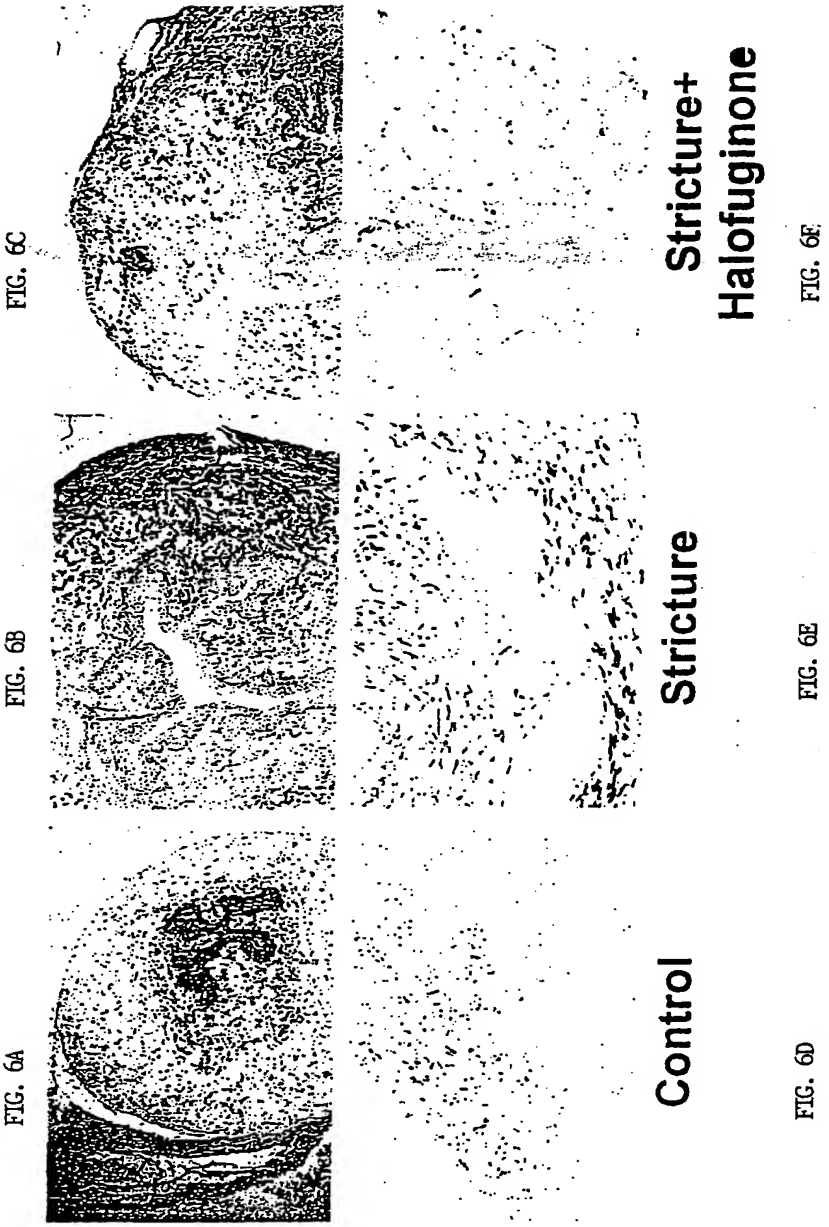


FIG. 6C FIG. 6F

FIG. 6B FIG. 6E

FIG. 6A FIG. 6D

FIG. 7A

FIG. 7B

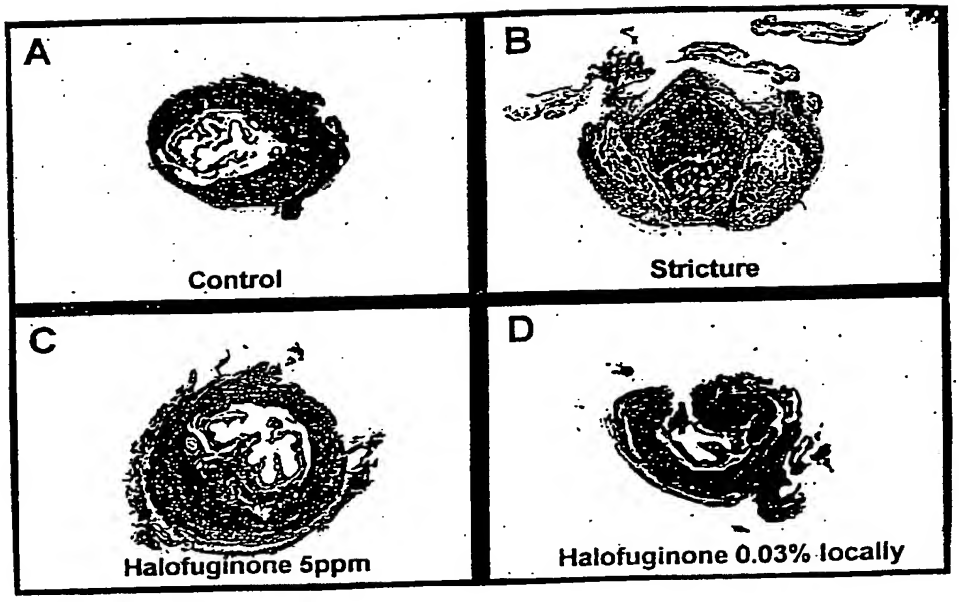


FIG. 7C

FIG. 7D

FIG. 8C

FIG. 8B

FIG. 8A

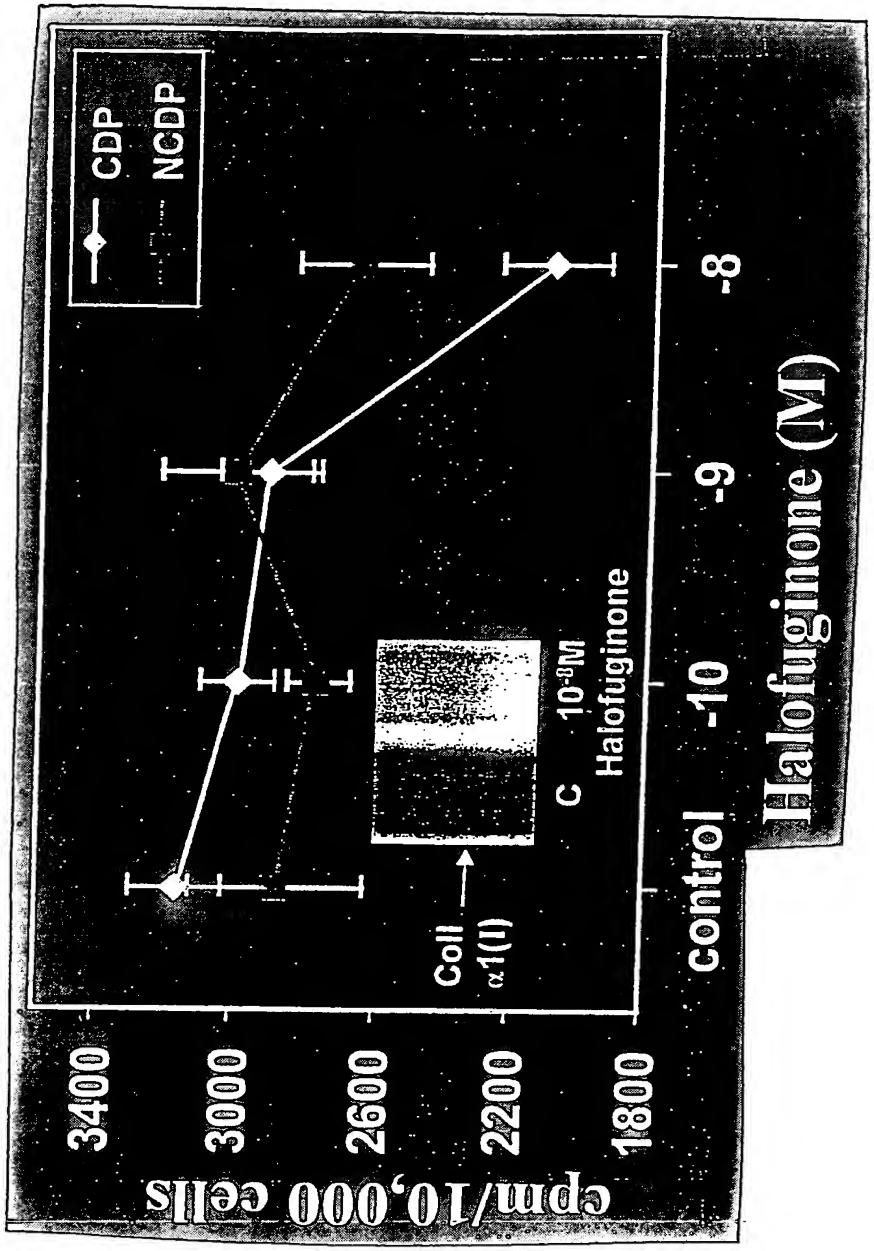

CDP
NCDP
cpm/10,000 cells
3400
3000
2600
2200
1800
Control
-10
-9
-8
Halofuginone (M)
C
10^{-8}M Halofuginone
Coll α1(I)

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL99/00441

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :A61K 31/505
US CL :514/259
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/259

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - A	US 5,891,879 A (NAGLER et al.) 06 April 1999 (6/4/99), see the entire document, especially column 3, lines 3-20.	1-11, 18 --- 12-17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
03 MAY 2000	13 JUN 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer FREDERICK KRASS Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)*

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 959/39

Box No. I TITLE OF INVENTION

PROMOTION OF WOUND HEALING

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Hadasit Medical Services and Development Company Ltd.
Kiryat Hadassash
Jerusalem 91120
Israel

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: IL

State *(that is, country)* of residence: IL

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

PINES, Mark
128 Pinsker St.
Rehovot 76308
Israel

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IL

State *(that is, country)* of residence: IL

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

FRIEDMAN, MARK M
Beit Samueloff
7 Haomanim Street
67897 Tel Aviv
Israel

Telephone No.
972-3-5625553

Facsimile No.
972-3-5625554

Teleprinter No.

☐ **Adress for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

VLODAVSKY, Israel
34 Arbel St.
Mevasereet Zion 90805
Israel

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IL

State *(that is, country)* of residence: IL

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

NAGLER, Arnon
46 Sderot Herzl
Jerusalem 74381
Israel

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IL

State *(that is, country)* of residence: IL

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

☒ AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ EP European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ AE United Arab Emirates
☒ AL Albania .....
☒ AM Armenia .....
☒ AT Austria .....
☒ AU Australia .....
☒ AZ Azerbaijan
☒ BA Bosnia and Herzegovina .....
☒ BB Barbados
☒ BG Bulgaria .....
☒ BR Brazil .....
☒ BY Belarus .....
☒ CA Canada
☒ CH and LI Switzerland and Liechtenstein
☒ CN China .....
☒ CU Cuba .....
☒ CZ Czech Republic .....
☒ DE Germany .....
☒ DK Denmark .....
☒ EE Estonia .....
☒ ES Spain .....
☒ FI Finland .....
☒ GB United Kingdom
☒ GD Grenada
☒ GE Georgia .....
☒ GH Ghana .....
☒ GM Gambia
☒ HR Croatia .....
☒ HU Hungary .....
☒ ID Indonesia
☒ IL Israel .....
☒ IN India .....
☒ IS Iceland
☒ JP Japan .....
☒ KE Kenya .....
☒ KG Kyrgyzstan .....
☒ KP Democratic People's Republic of Korea .....
☒ KR Republic of Korea .....
☒ KZ Kazakhstan .....
☒ LC Saint Lucia
☒ LK Sri Lanka
☒ LR Liberia
☒ LS Lesotho .....
☒ LT Lithuania
☒ LU Luxembourg
☒ LV Latvia
☒ MD Republic of Moldova .....
☒ MG Madagascar .....
☒ MK The former Yugoslav Republic of Macedonia .....
☒ MN Mongolia
☒ MW Malawi .....
☒ MX Mexico .....
☒ NO Norway
☒ NZ New Zealand .....
☒ PL Poland .....
☒ PT Portugal .....
☒ RO Romania
☒ RU Russian Federation .....
☒ SD Sudan
☒ SE Sweden
☒ SG Singapore
☒ SI Slovenia .....
☒ SK Slovakia .....
☒ SL Sierra Leone .....
☒ TJ Tajikistan .....
☒ TM Turkmenistan .....
☒ TR Turkey .....
☒ TT Trinidad and Tobago .....
☒ UA Ukraine .....
☒ UG Uganda .....
☒ US United States of America .....
☒ UZ Uzbekistan .....
☒ VN Viet Nam .....
☒ YU Yugoslavia .....
☒ ZA South Africa .....
☒ ZW Zimbabwe .....

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

☐ .....
☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1)				
item (2)				
item (3)				

☐ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): _____

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA /

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	: 5
description (excluding sequence listing part)	: 21
claims	: 5
abstract	: 1
drawings	: 9
sequence listing part of description	:
Total number of sheets	: 41

This international application is **accompanied by** the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*:

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: ENGLISH

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Mark M Friedman
agent

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ US

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference: 959/39
International application No.: IL99/00441	International filing date *(day/month/year)*: 13 AUG 1999	(Earliest) Priority date *(day/month/year)*:

Title of invention: PROMOTION OF WOUND HEALING

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

HAdasit Medical Services and Development Company Ltd.
Kiryat Hadassah
Jerusalem 91120
Israel

Telephone No.:

Facsimile No.:

Teleprinter No.:

State *(that is, country)* of nationality: IL

State *(that is, country)* of residence: IL

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

PINES, Mark
12B Pinsker St.
Rehovot 76308
Israel

State *(that is, country)* of nationality: IL

State *(that is, country)* of residence: IL

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

VLODAVSKY, Israel
34 Arbel St.
Mevasereet Zion 90805
Israel

State *(that is, country)* of nationality: IL

State *(that is, country)* of residence: IL

[X] Further applicants are indicated on a continuation sheet.

Sheet No. 2

International application No. IL99/00441

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

NAGLER, Arnon
46 Sderot Herzl
Jerusalem 74381
Israel

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
IL	IL

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

☐ Further applicants are indicated on another continuation sheet.

Sheet No. 3

International application No. IL99/00441

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

FRIEDMAN, Mark M.
c/o CASTORINA, Anthony
2001 Jefferson Davis Highway, Suite 207
Arlington, Virginia 22202
US

Telephone No.: 703-4151581

Facsimile No.: 703-5154864

Teleprinter No.:

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of:**

   [ ] the international application as originally filed

   the description [ ] as originally filed
   [ ] as amended under Article 34

   the claims [ ] as originally filed
   [ ] as amended under Article 19 (together with any accompanying statement)
   [ ] as amended under Article 34

   the drawings [ ] as originally filed
   [ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: .....

[ ] which is the language in which the international application was filed.

[ ] which is the language of a translation furnished for the purposes of international search.

[ ] which is the language of publication of the international application.

[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects **all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes **not to elect:**

Sheet No. 4

International application No.
IL99/00441

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:			For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:	sheets	☐	☐
2. amendments under Article 34	:	sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:	sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:	sheets	☐	☐
5. letter	:	sheets	☐	☐
6. other *(specify)* Change of Address	: 1	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ nucleotide and or amino acid sequence listing in computer readable form
6. ☐ other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

Mark M. Friedman
agent

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

CHAPTER II

PCT

FEE CALCULATION SHEET

Annex to the Demand for international preliminary examination

International application No.	IL99/00441
Applicant's or agent's file reference	959/39

For International Preliminary Examining Authority use only

Date stamp of the IPEA

Applicant Hadasit Medical Services and Development Company Ltd.

Calculation of prescribed fees

1. Preliminary examination fee	490	P
2. Handling fee *(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)*	137	H
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box	627 TOTAL	

Mode of Payment

[X]	authorization to charge deposit account with the IPEA (see below)	[ ]	cash
[ ]	cheque	[ ]	revenue stamps
[ ]	postal money order	[ ]	coupons
[ ]	bank draft	[ ]	other *(specify)*:

Deposit Account Authorization *(this mode of payment may not be available at all IPEAs)*

The IPEA/ US [X] is hereby authorized to charge the total fees indicated above to my deposit account.

[X] *(this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

06-2140		
Deposit Account Number	Date *(day/month/year)*	Signature